WO 2005/045014

# FIG. 1 OB Stock Virus



OB original source: Larvae or cell culture cloned



**Larvae** 1.7x10<sup>10</sup> OB per larvae



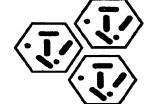
OB Master Stock 10<sup>11</sup> OB Stable at 4 °C for 10 years









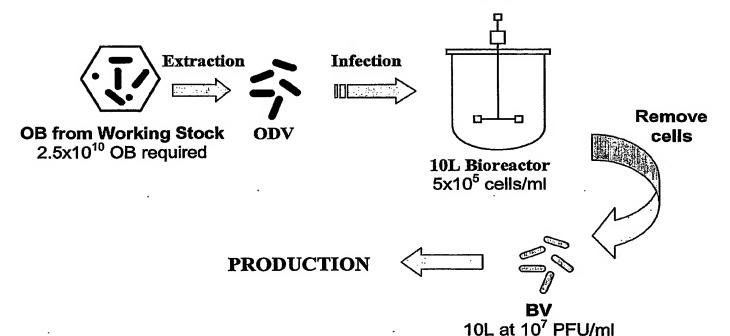


OB from Master Stock 30 larva required per year

OB Working Stock
5x10<sup>11</sup> OB
(sufficient for 20 runs/year)

Stable at 4°C for 1 week while titre

### **ODV** Extraction per run



WO 2005/045014 PCT/AU2004/001549

FIG. 2

#### **VPM3 MEDIA FORMULATION**

#### **SALTS**

Component	VPM 3 (MG/L)	VPM* (MG/L)
CaCl <sub>2</sub>	500	500
CoCl <sub>2</sub> .6H <sub>2</sub> O	0.05	0.05
CuCl <sub>2</sub> .2H <sub>2</sub> O	0.20	0.20
FeSO <sub>4</sub> .7H <sub>2</sub> O	1.70	1.70
KCI	1,200	1,200
MgSO <sub>4</sub>	918	918
MnCl <sub>2</sub> .4H <sub>2</sub> O	0.02	0.02
NaCl	2,700	2,700
NaHCO₃	350	350
NaH <sub>2</sub> PO <sub>4</sub> .H <sub>2</sub> O	1,160	1,160
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> .4H <sub>2</sub> O	0.04	0.04
ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.04	0.04

#### **SUGARS**

Component	VPM 3 (MG/L)	VPM* (MG/L)
Glucose	8,000	8,000
Sucrose	3,000	3,000
Maltose	500	500
Trehalose *	500	0
Galactose *	300	0

#### **AMINO ACIDS**

Component	VPM 3 (MG/L)	VPM* (MG/L)
L-Cystine.2HCl	200	200
L-Lysine.HCl	300	300
L-Methionine	200	200
L-Asparagine	300	300
L-Glutamic Acid (Na)	3,000	3,000
L-Glutamic Acid (K)	3,000	3,000
Hy Pep Dev 4602	750	750

#### **VITAMINS**

WO 2005/045014 PCT/AU2004/001549

Component	VPM 3 (MG/L)	VPM* (MG/L)
Inosine	200	200
Choline Chloride	10	10
Vitamins IPL-41 (100X)	10 ml	10 ml

#### **HYDROLYSATES**

Component	VPM 3 (MG/L)	VPM* (MG/L)
Yeast Extract	3,000	3,000
Primatone	2,500	2,500
Hy Soy	500	500
Casein	500	500
Lactalbumin (Edamin S)	500	500

#### **OTHER COMPONENTS**

Component	VPM 3 (MG/L)	VPM* (MG/L)
Dextran T 10 *	50	0
Chitosin *	2.5	0
Glutathione (Reduced Na)	.10	10
Glycerol	2,000	2,000

#### **LIPIDS**

Component	VPM 3 (MG/L)	VPM* (MG/L)
Cholesterol	4.5	4.5
Cod Liver Oil	12.5	12.5
Vitamin E acetate	3	3
Tween 80	25	25
Lecithin (Soya) *	4	0
ETOH (ml)	1.25	1.25
Pluronic Polyol F-68	900	900

## Specifications: pH: 6.2-6.3

Osmolarity: 355-375 mOsm/kg

VPM3 and VPM\* are low cost serum-free media that we have trialled for the ODV extraction process and subsequent passages. These media are similar but not identical to baculovirus/insect cell culture media reported in the literature. The additives indicated with an asterisk are unique additives by us into VPM3. VPM3 works better than VPM\* for the extraction process. Further optimisation of the media for the ODV extraction process is possible.

FIG. 3

Table 1: Virus yield data of different ODV extractions

Number of OB used for extraction	Yield at passage 4 (OB per cell)
5x10 <sup>9</sup> (100 OB per cell)	283
2.5 x10 <sup>9</sup> (50 OB per cell)	352
1x10 <sup>9</sup> (20 OB per cell)	369
5x10 <sup>8</sup> (10 OB per cell)	339
2.5 x10 <sup>8</sup> (5 OB per cell)	383

WO 2005/045014 PCT/AU2004/001549

FIG.4

